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Standard Review

Cationic lipids used in non-viral gene delivery systems

Sarder Nasir Uddin

Biotechnology and Genetic Engineering Disciplne, Khulna University, Khulna-9208, Bangladesh. E-mail: opunasir@gmail.com.

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To perform gene transfer two types of vectors are available, (i) viral vectors (ii) nonviral vectors. Among the vectors, nonviral vectors have proved less toxic and safe compare to viral vectors through clinical trials. No single vector is proved suitable for every gene transfection experiment. Cationic lipids are experimentally established as non-viral vector with higher transfection efficiency. Identifying the barriers for transfection and the possible solutions, stability improvement research is needed for better clinical performance of cationic vectors. The newly described liposomal preparation, Liposomes-Protamine-DNA (LPD), has shown superiority over conventional Liposomes-DNA complexes (lipoplexes). In future it is also possible to find new conjugates as cationic lipids bearing many properties, suitable to bound with DNA and penetrate cell. Considering all these properties, these works reviews the most recent studies highlighting cationic lipids used in nonviral gene delivery systems.

Key words: Gene therapy, non-viral, lipids, cation, delivery systems.

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INTRODUCTION

General aspects

Gene therapy represents a new and promising therapeutic modality. The underlying principle is based on the introduction of genetic material into cells to generate a curative biological effect (Miller, 1992; Mulligan, 1993). Gene therapy is not limited to hereditary diseases but can be used for a broad variety of different acquired diseases, such as infections, degenerative disorders, and cancer. The most challenging issues for successful application ofgene therapy to human diseases concern are

(1) The choice of the relevant therapeutic gene.
(2) The choice of promoter and regulatory sequences driving expression of the transgene.
(3) The vector used for delivery of the transgene into target cells.

Promoter, regulatory elements and vector characteristics determine transduction efficacy (the number of target cells expressing the transgene and the intensity of gene expression per cell), specificity of the transduction, time of transgene expression, the host's immune response against the vector, and eventually undesired side effects. At present, efforts are being focused on the search for vectors with less toxicity and prolonged and controlled

transgene expression, thereby widening the potential application of gene therapy to a high spectrum of medical fields. To perform gene transfer two types of vectors are available: viral vectors (retorviruses, adeneviruses, herpesviruses, lentiviruses and hybrid/ retoradeno viruses etc), and nonviral vectors (cations e.g. polymers and lipids, naked DNA, artificial chromosomes, plasmid, conjugates and complexes) (Uddin and Islam, 2006).

Despite the impressive progress in biomedical sciences during the last decades, the therapy of many liver diseases remains unsatisfactory. This applies not only to primary and metastatic liver tumours, hepatic cirrhosis, and hereditary metabolic diseases but also to a high proportion of cases with chronic viral hepatitis that do not respond to current antiviral therapy. There is evidently an urgent need for efficient alternative therapeutic approaches. In recent years gene therapy has emerged as a new and promising method to treat human diseases (Schmitz et al., 2005)

The genetic materials used for gene therapy are natural or chimeric genes (that direct the synthesis of therapeutic proteins inside the transduced cells) or subgenomic DNA and RNA molecules (that act directly to modify expression of endogenous genes). Natural genes are genomic sequences that encode natural proteins such as enzymes, costimulatory molecules, antigens etc. Chimeric genes are genomic constructs that do not exist in nature that encode for such molecules as transdominant negative proteins or single chain antibodies (Herskowitz, 1987).

Subgenomic DNA and RNA sequences include ribozymes, antisense molecules, and RNA decoys, among others. A ribozyme is an RNA molecule that hybridizes specifically with a messenger RNA and possesses a catalytic active domain which cleaves the target mRNA (Haseloff et al., 1988; Symons and Ribozymes, 1994). Antisense molecules are DNA or RNA constructs which bind complementary sequences by hybridization and interfere with pre-mRNA processing and messenger RNA translation and accelerate mRNA degradation (Crooke, 1998; Eguchi, 1991). RNA decoys are RNA sequences, which ligate transactivating proteins as a substitute for their original binding domain, thus, blocking the function of the regulatory proteins (Sullenger et al., 1991).

Gene therapy vectors

Gene therapy vehicles can be categorized into two groups: biological and non-biological systems. Each group has its own advantages and limitations. The main objective in gene therapy is the development of efficient, non-toxic gene carriers that can encapsulate and deliver foreign genetic materials into specific cell types. During the past two decades, enormous research in the area of gene delivery has been conducted worldwide, in particular for cancer gene therapy application (Uddin and Islam, 2006). Viral vectors are biological systems derived from naturally evolved viruses capable of transferring their genetic materials into the host cells. Many viruses including retrovirus, adenovirus, herpes simplex virus (HSV), adeno-associated virus (AAV) and pox virus have been modified to eliminate their toxicity and maintain their high gene transfer capability. The limitations associated with viral vectors, however, in terms of their safety, particularly immunogenicity, and in terms of their limited capacity of transgenic materials, have encouraged researchers to focus on non-viral vectors as an alternative to viral vectors. Non-viral vectors are generally cationic in nature. They include cationic polymers such as polyethylenimine (PEI) and poly L-lysine (PLL), cationic peptides and cationic liposomes. The newly described liposomal preparation LPD (liposomes/protamine/DNA), for example, has shown superiority over conventional liposomes/DNA complexes (lipoplexes) (Aneed, 2004). However, physical properties such as size and zeta potential play a critical role in their efficiency. In either delivery system, selected modifications that can produce safe efficient and targetable gene carriers are desirable. Although non-viral vectors are less efficient than viral ones, they have the advantages of safety, simplicity of preparation and high gene encapsulation capability. This article reviews the most recent studies highlighting the advantages and the limitations of nonviral cationic types of gene delivery systems used in gene therapy (Aneed, 2004).

Both viral and non-viral vectors are used to transfer genetic material to the inside of target cells. In general, non-viral vectors have lower transduction efficiency and allow for shorter duration of transgene expression than viral vectors. The ideal vector should have;

(i) Low antigenic potential, high capacity, high transduction efficiency.
(ii) Allow controlled and targeted transgene expression.
(iii) Reasonable expense.
(iv) Safe for both patient and the environment.

Gene delivery vehicles must be selected according to the specific therapeutic aim. But the perfect vector covering all therapeutic and safety requirements does not exist and much work is needed in this important research field (Prince, 1998).

Non-viral vectors

Non-viral systems are cationic in nature. They interact with negatively charged DNA through electrostatic interactions. The total charge however maintains a positive net value. This will enable the carrier of efficiently interacting with the negatively charged cell membranes and internalizes into the cell, which occurs mainly through the endocytosis pathway (Behr, 1994). The cationic nature of the non-viral vectors helps them to interact with

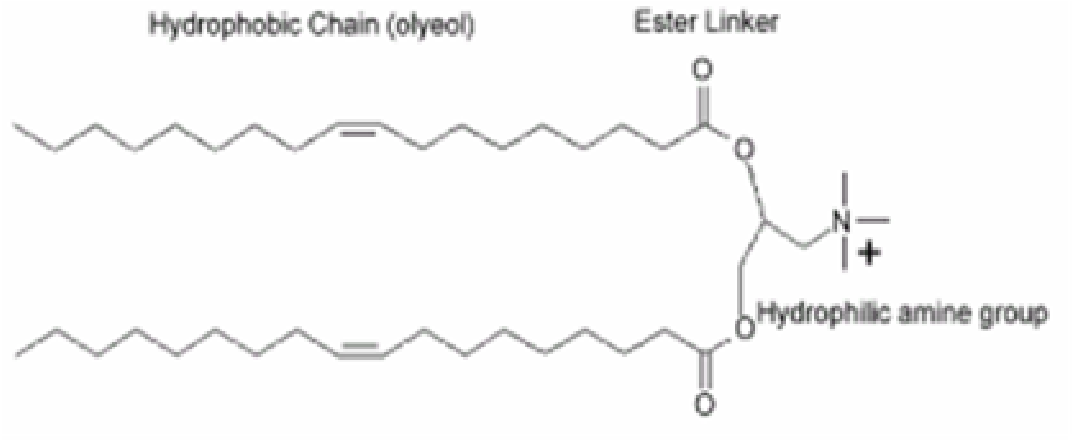

Figure 1. Schematic representation of the cationic lipid DOTAP. The hydrophobic part (olyel), the ester linker and the hydrophilic portion are presented. Most other cationic lipids share the same general structure (Aneed, 2004).

the negatively charged DNA through electrostatic interactions. The reaction complex also bears positive net values, which enable the carrier to react efficiently with negatively charged cell membranes. Nonviral gene transfer vectors have been actively studied in the past years in order to obtain structural entities with minimum size and defined shape. The final size of a gene transfer vector, which is compacted into unimolecular complexes, is directly proportional to the mass of the nucleic acid to be compacted. Expression of compacted ssDNA was observed in hepatoma cell lines. Firstly, galactosylated ssDNA complexes were successfully delivered into cells and then expression of the asialoglycoprotein receptor via receptor-mediated endocytosis. The reduced size and biophysical behaviour of ssDNA vectors may provide an advantage for transfection of eukaryotic cells (Behr, 1994).

Cationic lipids

Since their introduction as gene carriers in 1987 (Felgner et al., 1987). Liposomes have become one of the most studied non-viral vectors. They include a group of positively charged lipids at the physiological pH. As other nonviral vectors, they interact with the negatively charged DNA through electrostatic attractions. Cationic lipids were used mainly in the form of liposomes. More recently, however, cationic lipid emulsions have been described and evaluated as possible non-viral gene carriers (Yi et al., 2000; Choi et al., 2002). In a variety of cationic lipids synthesized, DC-Chol, *N*-[1-(2,3-dimyristyloxy)propyl]-*N, N*-dimethyl-*N*-(2-hydroxyethyl) ammonium bromide and N,N,N-trimethyl-2-bis[(1-oxo-9-octadecenyl)oxy]-(Z,Z)-1propanaminium methyl sulfate have been used in human clinical trails by local injection (Hara et al., 2003).

Liposomes

Basic nature and transfection mechanism

Cationic lipids are amphiphilic molecules composed of one or two fatty acid side chains (acyl) or alkyl, a linker and a hydrophilic amino group**.** The example of cationic lipid 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) Figure 1, which consists of two unsaturated diacyl side chains (oleoyl), ester linker and propyl ammonium group (Lasic, 1997). Other lipids may contain different linkers such as the more stable ether linkages, e.g. N [1-(2,3-dioleyloxy) propyl]- N,N,N-trimethylammonium chloride (DOTMA)(Mayer et al. 1986). The hydrophobic part can also be cholesterol derived moieties (Gao and Hui, 2001). Cationic liposomes spontaneously interact with the negatively charged DNA to form a stable complex that promotes the gene transfer to cells. The mode of formation and the size of cationic liposomes/DNA complexes were investigated using the atomic force microscopy (AFM). Also the most important physical-chemical factors involved in cationic liposome-mediated gene transfection, e.g. size and lipidic composition, were evaluated through the transfection of complexes with different liposomes/DNA molar ratio into three types of cultured cells. Cationic liposomes, composed of a neutral lipid (phosphatidilcoline), a cationic lipid dimethyldioctadecylammonium bromide (DDAB), a co-lipid-1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine (DOPE) and a phospholipid derivative of polyethylene glycol (DSPE-mPEG) at different molar ratio, were mixed with a plasmid pCMVbeta to form liposomes/DNA complexes.

In 2003, Ruozi et al. have demonstrated that the complexes were made by complicated structures in which the liposomes tend to aggregate and the DNA is surrounded by lipidic material. *In vitro* transfection efficiency

by liposomes/plasmid pCMVbeta complexes was found to depend on the kind of lipid associated in the liposomes and the liposomes/DNA mixing ratio. The importance of associating DOPE in cationic liposomes was confirmed; this co-lipid is able to improve the ability of cationic liposomes to transfect cells but in addition, the AFM images and the EtBr fluorescence experiments have suggested that this lipid can also play an important role to facilitate the formation of stable liposomes, which efficaciously protect the DNA by nuclease digestion (Ruozi et al., 2003).

In aqueous media, cationic lipids are assembled into a bilayer vesicular-like structure (liposomes). Liposomes are first arranged into multilamellar vesicles. Unilamellar vesicles can then be obtained by sonication (Huang, 1969), detergent removal (Jiskoot et al., 1986) or extrusion through porous membranes (Hope et al., 1985; Mayer et al., 1986). Liposomes/DNA complex is usually termed a lipoplex. Based on freeze-fracture electron micrographs and x-ray diffraction studies, it was suggested that DNA is sandwiched between many liposomal particles (Radler et al., 1997; Sternberg et al., 1994). This structure is in agreement with the increase of particle size after the addition of DNA to cationic liposomes (Almofti et al., 2003). Negatively charged DNA will neutralize cationic liposomes resulting in aggregation and continuous fusion with time while DNA being entrapped during this process. Because of poor stability (that is, continuous aggregation), lipoplexes are usually administered directly after their formation. Many physical factors influence stability, complex formation and transfection efficiency of lipoplexes such as particle size, zeta potential, DNA/liposomes ratio and ionic strength of the medium (Almofti et al., 2003). Producing the favorable, stable, small lipoplex particles was obtained with the development of the novel liposomal formulation liposomes/protamine/DNA (LPD), are discussed as follows.

Liposome mediated gene therapy in kidney

Among non-viral vectors, the liposome system is a promising procedure for kidney-targeted gene therapy. Using cationic liposome, tubular cells were effectively transduced by retrograde injection of liposome/cDNA complex (U). Although transgene expression was reportedly modest using cationic liposomes, this method improved renal disease models such as carbonic anhydrase II deficiency and unilateral ureteral obstruction (U). In contrast, HVJ-liposome system is an effective transfection method to glomerular cells using intra-renal arterial infusion and improved glomerular disease models such as glomerulonephritis and glomerulosclerosis. In addition, intra-renal pelvic injection of DNA by HVJ-liposome system showed transgene expression in interstitial fibroblasts. In kidney-targeted gene therapy, liposome-mediated gene transfer is an attractive method because of its simplicity and reduced toxicity. In spite of modest transgene expression, several renal disease models were successfully modulated by liposome system (U). Although one limitation of liposome-mediated gene delivery is the duration of transgene expression, the liposome/cDNA complex can be repeatedly administered due to the absence of an immune response (Ito et al., 2003).

Transfer of single gene vs multiple genes:

Liposomal gene transfer is an effective therapeutic approach for the treatment of several pathophysiologic states. In 2004, Jeschke and Klein showed that exogenous gene transfer of multiple cDNA sequences have an additive effect on intracellular and biological responses when compared to the same gene administered as a single cDNA sequence. Our findings demonstrate that gene therapy with multiple genes is feasible, and that the gene transfer of multiple genes can enhance and accelerate physiologic and biological effects (Jeschke and Klein, 2004).

Transfer condition with extracellular factors

Liposome-mediated adenomatous polyposis coli (APC) to the duodenum is feasible even in the presence of bile and varying pH, raising the potential of future gene therapy for this extremely difficult to treat condition (Lee et al., 2004). Cationic polymers and liposomes are used to warp DNA into complexes that promote its cellular uptake. Polyanionic glycosaminoglycans (GAGs) on the cell surface interact with the cationic DNA complexes and influence transfection. The uptake of DNA complexes varies depending on carrier, cell type and amounts of cell surface heparan sulfate (HS), chondroitin sulfate (CS) and hyaluronan (HA) where as GAGs inhibit the transgene expression. This implies that cell-surface GAGs probably direct complexes into intracellular compartments that do not support transcription (Ruponen et al., 2004).

Liposome complexes

Reconstituted chylomicron remnants (RCR): Lipoproteins are naturally occurring biological emulsions and serve as carriers of cholesterol and other lipids in systemic circulation. Dietary lipids absorbed by the intestine are packed into triglyceride-rich lipoproteins termed chylomicrons. In the blood circulation, chylomicrons are remolded to chylomicron remnants by hydrolysis of the core triglycerides by lipoprotein lipase and by accepting apolipoproteins. Finally, the remnants are taken up by liver parenchymal cells via apolipoprotein-specific receptors (Hong et al., 2004).

Recently reconstituted chylomicron remnants (RCR) were established using commercially lipids and shown to be taken up by hepatocytes following intravenous injection. If the therapeutic DNA can be incorporated into the RCR, the resulting nonviral particles could be an efficient vector for hepatic gene therapy. The incorporation of DNA into RCR by means of the formation of a hydrophobic DNA complex with a quaternary ammonium derivative of cholesterol is already reported. Intraportal injection of the resulting particle leads to highly efficient expression of foreign genes into the liver of mice. The therapeutic potential of the novel vector is demonstrated using human 1-antitrypsin (hAAT) gene and its production in the liver (Hara et al., 2003).

Tat/liposome/DNA (TLD)

HIV-1 Tat protein transduction domain (PTD) peptide facilitates gene transfer in combination with cationic liposomes. Incorporation without covalent linkage of a 17-amino acid PTD peptide into gene delivery lipoplexes improves gene transfer. Tat/liposome/DNA (TLD) enable transfection of highly recalcitrant primary cells in the form of air/liquid interface cultures of sheep tracheal epithetlium. Treatment with chloroquine increased, and incubation at low temperature decreased, TLD transfection (Hyndman et al., 2004).

DOTAP, DOPE and cholesterol (DDC): A new ternary cationic liposome formulated with dioleoyl trimethylammonium propane (DOTAP), 1,2-dioleoyl-3-phosphatidylethanolamine (DOPE) and cholesterol (Chol) (DDC), It has been recently found to have a selective high gene transfer ability in ovarian cancer cells. The factors controlling cationic lipid/DNA (CL-DNA) complexes-mediated gene transfer depend not only on the formulations of the cationic liposomes and their thermodynamic phase, but also significantly on the cell properties (Caracciolo et al., 2003). In an experiment, wild-type p53 DNA was transfected into the ovarian cancer cells, using the DDC as a nonviral vector and the expression and activity of p53 gene were evaluated both *in vitro* and *in vivo*. DDC liposomes were prepared by mixing DOTAP: DOPE: chol in a 1:0.7:0.3 molar ratios using the extrusion method. Plasma DNA (pp53-EGFP) and DDC complexes were transfected into ovarian carcinoma cells (OVCAR-3 cells) and gene expression was determined by reverse transcription-polymerase chain reaction and western blot analysis. The transcription of liposome complexed p53 gene resulted in a high level of wild-type p53 mRNA and protein expression OVCAR-3 cells. The DDC-mediated p53 DNA delivery may have the potential for clinical application as nonviral vector-mediated ovarian cancer therapy due to its effective induction of apoptosis and tumor growth inhibition (Kim et al., 2003).

Anionic liposomal vectors

Anionic liposomal vectors based on the composition of retroviral envelops (artificial viral envelops, AVEs) can be used for transgene expression in many cell lines. AVE liposomes, dispersed in 10% serum containing growth medium, efficiently delivered plasmid DNA to HuH-7 (Human hepatoma cell line) cells. Small unilamellar AVE vesicles containing 15 mol % digalactosyl diglyceride (DGDG) are efficiently targeted to the liver via the hepatic asialoglycoprotein receptor (Mady et al., 2004).

Structure and activity relationship

Despite the numerous studies that focus on the possible relationship between cationic lipid structure/composition and lipoplex transfection activity, solid conclusions are rarely obtained. This is mainly due to the complex factors affecting gene transfer. The levels of transfection among different cell lines, for example, may significantly vary with the same lipoplex formulations (Zou et al., 2004; Balasubramaniam et al., 1996). More important, the correlation between *in vitro* and i*n vivo* experiments is not always obtained (Gorman et al., 1997; Solodin et al., 1995). Therefore, it is expected that empirical findings will remain the main source for structure/activity relationships. It has, however, been proven that the addition of neutral lipids (colipids) will increase the transfection ability of lipoplexes both *in vitro* and *In vivo*. The most used colipids are cholesterol (Liu et al., 1997; Bennett et al., 1995) and dioleylphos-phatidylethanolamine (DOPE) (Hui et al., 1996; Farhood et al., 1995). While the former is more efficient i*n vivo*, the latterenhances *in vitro* lipoplex transfection (Simberg et al., 2001; Hong et al., 2004). It was demonstrated that lipoplexes enter cells through endocytosis and fusion pathways (Farhood et al., 1995). Neutral lipids facilitate conformational changes from a bilayer structure into hexagonal arrangement at the endosomal level (Hafez et al., 2001). This change will trigger the release of the encapsulated DNA into the cytoplasm and not reaching the destructive lysosomal environment.

Liposomes-protamine-DNA (LPD)

Protamine is arginine-rich peptide, which can condense negatively charged DNA before being complexed with cationic lipids. The polycation PLL (which is less efficient) was also tested for LPD preparation in early studies (Gao and Huang, 1996).

Liposomes will interact with condensed DNA resulting in lipid rearrangement and the formation of compact liposomes/ DNA complex (LPD) (Li and Huang, 1999). In comparison of proposed complex structures between LPD and conventional lipoplexes (Figure 2). DOTAP and

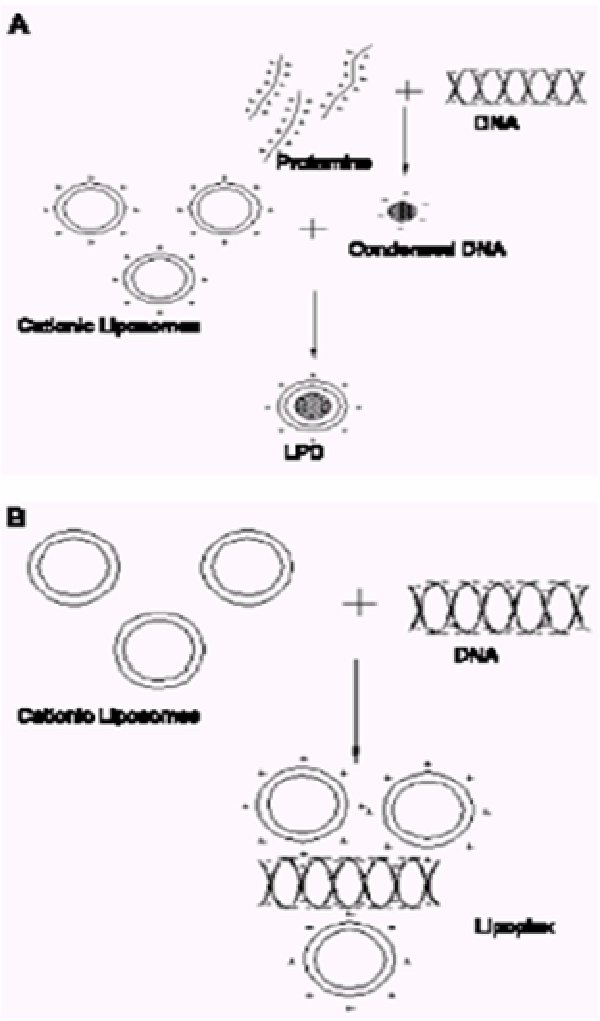

Figure 2. Schematic comparison between LPD and lipoplex particles. In (A): DNA is neutralized and condensed with the protamine before the formation of the complex LPD. In (B): DNA is sandwiched between liposomal particles forming the 'conventional' lipoplex. (Aneed, 2004).

3h-[N-(NV,NV-dimethylaminoethane)-carbamoyl]cholesterol (DC-Chol) were used for the preparation of LPD with the most common co-lipids: cholesterol and DOPE, respectively (Tan et al., 2002). Particle size distribution of LPD ranged from 100 to 250 nm, which is almost three to five times less than conventional lipoplexes (Gao and Huang, 1996; Ueno et al., 2002; Sorgi et al., 2001).

Stability with no compromise in the transfection ability was maintained for 4 months when LPD was stored at 4°C or at room temperature after lyophylization (freeze-drying or spray-drying) (Seville et al., 2002; Li et al., 2000). Both *in vitro* and *in vivo* studies showed superiority of LPD-mediated gene transfer over conventional liposomes (Sorgi et al., 2001; Li et al., 1998). It is believed that the small size of LPD will facilitate endocytosis and increase the *in vivo* circulating half life. Like other non-modified liposomes, LPD tends to accumulate preferably in the lungs after injection from the tail vein of a mouse (Li and Huang, 1999; Li et al. 1998). In addition to the lungs, reporter gene expression, however, was also detected in other organs such as the kidney, the spleen and the liver (Li et al., 1998; Li and Huang, 1997). For therapeutic applications, that is, administration of DOTAP: Chol LPD carrying tumor suppressor genes Rb or E1A in cancer animal models resulted in apoptosis induction, tumor size reduction and life span increase in the treated animals (Ueno et al. 2002; Nikitin et al. 1999). As expected, antitumoral synergistic effects were obtained when E1A LPD treatment was combined with the chemotherapeutic agent paclitaxel (Lee et al. 1996).

For targeting purposes, LPD was recently coated with the liver targeting ligand asialofetuin through charge–charge interactions, which significantly increases HepG2, liver cancer cells, uptake of the encapsulated DNA (Arangoa et al., 2003). LPD composed of DC-Chol/DOPE was also tested in clinical settings when two children with Canavan disease (a fatal CNS disease characterized by spongy degradation of cerebral white matter) were treated with ASPA gene via intracerebral application; both subjects showed some clinical improvements (Leone et al., 2000).

LID vector complex

An integrin-targeted synthetic vector system was developed for the transfection of haematopoietic cell lines and dendritic cells. The vector consists of a cationic liposome, Lipofectin (L), a peptide that both targets integrins and binds to DNA (I) and plasmid DNA (D). These components interact electrostatically to form the LID vector complex. Targeted gene delivery was demonstrated by comparing transfected luciferase reporter gene levels using LID complexes containing integrin-targeting peptide sequences with a control peptide. Under optimized conditions transfection efficiencies of 19% for TF-1 cells, 28% for Jurkat cells and 10% for primary dendritic cells were achieved. The LID vector may thus find application for gene-transfer experiments in haematopoietic cell lines and for the development of genetic vaccines using transfected dendritic cells (Uduehi et al., 2003).

Solid lipid nanoparticles (SLN)

Cationic solid lipid nanoparticles (SLN) for gene transfer are formulated using the same cationic lipids as for liposomal transfection agents. SLN is smaller in diameter than the corresponding liposomes (88 vs.148 nm) and atomic force microscopy (AFM) supported some structu-

ral differences. Cationic lipid composition seems to be more dominant for *in vitro* transfection performance than the kind of colloidal structure it is arranged in. Hence, cationic SLN extend the range of highly potent non-viral transfection agents by one with favourable and distinct technological properties (Tabatt et al., 2004).

Peptide mediated gene transfer: Ligand-targeted plasmid based gene delivery systems was developed for gene transfer to tumor endothelium. The single and double disulfide lipopeptides were then tested for gene transfer to HUVECs (human umbilical derived endothelial cell) using DOTMA: Cholesterol cationic liposomes. The polyplexes were formed by rapidly mixing plasmid DNA with DOTMA:CHOL liposomes at a 3:1 charge ratio in 2% ethanol, 10% lactose. The ethanol was removed by lyophilization and upon rehydration; the lipoplexes had a mean diameter of approximately 100nm. HUVEC transfection studies showed that increasing the mol% of the single disulfide RGD lipopeptide to 20 mol% increased gene transfer by 10 fold (Anwer et al., 2004).

Barriers of using cations in non viral gene therapy

Extracellular and intracellular barriers: Complexes of DNA with cationic lipids and cationic polymers are frequently used for gene transfer. Extracellular interactions of the complexes with anionic glycosaminoglycans (GAGs) may interfere with gene transfer. Interactions of GAGs with carrier DNA complexes have been studied using tests for DNA relaxation (ethidium bromide intercalation), DNA release (electrophoresis), and transfection (pCMVbGal transfer into RAA smooth muscle cells). Several cationic lipid formulations (DOTAP, DOTAP/Chol, DOTAP/DOPE, DOTMA/DOPE, DOGS) and cationic polymers (fractured dendrimer, polyethylene imines 25 and 800 kDa, polylysines 20 and 200 kDa) were tested. Polycations condensed DNA more effectively than monovalent lipids. Hyaluronic acid did not release or relax DNA in any complex, but it inhibited transfection by some polyvalent systems (PEI, dendrimers, DOGS). Gene transfer by other carriers was not affected by hyaluronic acid. Sulfated GAGs (heparan sulfate, chondroitin sulfates B and C) completely blocked transfection, except in the case of liposomes with DOPE. Sulfated GAGs relaxed and released DNA from some complexes, but these events were not prerequisites for the inhibition of transfection. Furthermore, preliminary results suggest that cell surface GAGs, particularly heap-ran sulfate, inhibit gene transfer by cationic lipids and polymers (Ruponen, et al. 2003).

The additional hydrogen bonding or covalent interactions of the head group with the plasmid DNA, leading to higher binding affinity of the cationic lipids to pDNA, results in higher transfection. This hypothesis is supported by TEM observations where elongated complexes were observed and more lipids were seen associated with the DNA (Narang et al., 2005).

Effect of human plasma proteins

In situ gene expression assay indicated that both the ratio of DNA and liposome and the dose of DNA could affect the gene transfection efficiency. Naked endostatin plasmid intratumoral injection can get similar gene transfection efficiency to liposome-DNA complex when used in situ (Ma, et al. 2004).

Interaction of cationic lipid/DNA complex with the plasma is a limiting step for the cationic lipid-mediated intravenous gene transfer and expression process. Most of the plasma components that interact with the complex and inhibit its transfection efficiency are still unknown. In an experiment, human plasma proteins and lipoproteins that bind to a cationic lipid/DNA complex were isolated on a sucrose density gradient and identified by 2-D gel electrophoresis. Protein binding did not result in complex dissociation or DNA degradation. The effects of several complex-binding plasma components on the transfection efficiency were studied using lung endothelial cells cultured *in vitro*. Lipoprotein particles caused a drastic loss of the transfection efficiency of the complex. Surprisingly, fibrinogen was found to activate the transfection process. The roles of these complex-binding plasma components on the complex uptake efficiency were quantitatively assessed using radiolabeled plasmid DNA and qualitatively evaluated using fluorescence microscopy. A good correlation was found between the effects of the complex-binding plasma components on the transfection and on cell uptake efficiencies. In contrast to what was generally believed, our data suggest that disruption of the complex does not occur when it is in contact with the plasma and therefore could not be responsible for the loss of transfection activity. Instead, coating of complexes with plasma components seems to be responsible for reduced uptake by cells, which in turn results in reduced transfection (Tandia et al., 2003).

Improvement of transfection efficiency

Efficiency increasing by surface coating: Systemic gene delivery systems are needed for therapeutic applications. For systemic circulation, masking the surface charge of DNA complexes has to be accomp-lished to avoid interactions with plasma components, erythrocytes and the reticuloendothelial system. Polyplexes based on polyethylenimine (PEI), shielded with polyethylene glycol (PGE) and linked to the receptor binding ligands transferrin (Tf) or epidermal growth factors (EGF) have been developed. Complexes were found to mediate efficient gene transfer into tumor cell lines in a receptor dependent and cell-cycle dependent manner. Systemic

administration of surface shielded Tf-PEI polyplexes into the tail vein of mice resulted in preferential gene into distantly growing subcutaneous tumors. In contrast, application of positively charged PEI polyplexes directed gene transfer primarily to the lung (Ogris and Wagner, 2002).

Delivery efficiency and gene expression efficiency increasing action

Tumor targeting is an important issue in cancer gene therapy. A gene transfection method, based on light inducible photochemical internalization (PCI) of a transgene, is already developed to improve gene delivery and expression selectively in illuminated areas, for example in tumors. PCI improve the non-viral vector polyethylenimine (PEI)-mediated transfection of therapeutic gene, the “suicide” gene encoding herpes simplex virus thymidine kinase (HSV+K). Photochemical transfection allows selective enhancement in gene expression and gene mediated biological effects (cell killing by the HSV+K/GCV approach) in response to illumination (Prasmickaite et al., 2004).

Systemic gene therapy vectors must be designed to safety and efficiently escort DNA from outside the cell to the nucleus and to overcome several physiological barriers that are obstacles to internalization, escape from endocytic vesicles, movement through the cytoplasm and transport into the nucleus. Chloroquine appears to facilitate PLL-mediated gene delivery by a mechanism other than buffering of endocytic compartments. Additionally, PEI does not appear to buffer endocytic compartments but requires exposure to an acidic environment for efficient gene delivery (Forrest and Pack, 2002).

Conclusion

Gene therapy is one of the youngest and most promising fields of medicine. In the course of one decade the basic research in recombinant DNA has been translated into a number of applied projects aiming at curing the diseases at its root, the gene. Though no clinically acceptable protocols have yet been developed, the researcher’s ability to solve many technical problems of gene therapy has been greatly improved. Several viral and nonviral gene therapy vectors have been evaluated for a range of conditions in animal models and in clinic. Both physical and immunological barriers exist, and these must be tackled. Cationic lipids showed a great potential among the vector implied for gene therapy, and it also proved that they are safer than viral vectors. Many types of cationic lipids are tested but it is still a vital field of research to invent new one.

From this review, it has emerged that a single vector is highly unlikely to be optimal for all gene therapy applications. To improve gene transfer efficiency, the appropriate vector must be selected for transfection of the required cell type. Ultimately all disease targets will benefit from an improved understanding of the biology of vector delivery, uptake and expression. Improvements in vector design will also reap rewards in the clinic. Research into vector design and targeting needs to proceed in parallel with early clinical studies, where proof of concept and identification of barriers to clinical gene transfer can inform the next step to basic research.

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